

**Figure 1. The amino acid sequences for the variable region of the heavy chain (VH) (A), and the light chain (VL)(B). CDR's are boxed.**

**(A) RFB4 VH sequence**

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E V Q L V E S G G G L V K P G G S L K L S C A A S G F A F S
  I Y D M S   W V R Q T P E K R L E W V A   Y I S S G G G T
T Y Y P D T V K G   R F T I S R D N A K N T L Y L Q M S S L
K S E D T A M Y Y C A R   H S G Y G S S Y G V L F A Y   W G
Q G T L V T V S A

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**(B) RFB4 VL sequence**

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D I Q M T Q T T S S L S A S L G D R V T I S C   R A S Q D I
S N Y L N   W Y Q Q K P D G T V K L L I Y   Y T S I L H S
G V P S R F S G S G S G T D Y S L T I S N L E Q E D F A T Y
F C   Q Q G N T L P W T   F G G G T K L E I K

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**Figure 2. A comparison of different human framework sequences to that of the RFB4. Amino acid that differs from the parent framework is shown in bold. The source of the human framework is indicated in parenthesis on the left of each framework. CDR's are boxed.**

**(A) VH**

	----- FR1 -----	
	E V Q L V E S G G G L V K P G G S L K L S C A A S G F A F S	
(EIK)	E V Q L V E S G G G L V - P G G S L <b>R</b> L S C A <b>T T</b> G F A F S	
(RF)	<b>Q</b> V <b>Q</b> L V E S G G G V V <b>Q</b> P G <b>R</b> S L <b>R</b> L S C A A S G F <b>S</b> F S	
(WAS)	E V Q L V E S G G G L V <b>Q</b> P G G S L <b>R</b> L S C A A S G F <b>S</b> F S	
	----- FR2 -----	
	<div style="border: 1px solid black; display: inline-block; padding: 2px;">I Y D M S</div> W V R Q T P E K R L E W V A <div style="border: 1px solid black; display: inline-block; padding: 2px;">Y I S S G G G T</div>	
	(WAS) W V R <b>Q</b> A P <b>G</b> K <b>G</b> L E W V A	
	----- FR3 -----	
	<div style="border: 1px solid black; display: inline-block; padding: 2px;">T Y Y P D T V K G</div> R F T I S R D N A K N T L Y L Q M S S L	
	(GAL) R F T I S R D N A K N <b>S</b> L Y L Q M N S L	
	----- FR3 -----	
	K S E D T A M Y Y C A R <div style="border: 1px solid black; display: inline-block; padding: 2px;">H S G Y G S S Y G V L F A Y</div> W G	
	R V E D T A L Y Y C A R (DOB) W G	
	----- FR4 -----	
	Q G T L V T V S A	
	<b>Q</b> G T L V T V S <b>T</b>	

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(B) VL

----- FR1 -----  
 D I Q M T Q T T S S L S A S L G D R V T I S C      R A S Q D I  
 (JOH) D I Q M T Q S P S S L S A S V G D R V T I S C

----- FR2 -----  
 S N Y L N      W Y Q Q K P D G T V K L L I Y      Y T S I L H S  
 (Vd'CL)      W Y Q Q K P G K A P K L L I Y

----- FR3 -----  
 G V P S R F S G S G S G T D Y S L T I S N L E Q E D F A T Y  
 (WES) G V P S R F S G S G S G T E F T L T I S S L Q P E D F A T Y

----- FR4 -----  
 F C      Q Q G N T L P W T      F G G G T K L E I K  
 F C      (RZ)      F G G G T K V E I K

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Figure 3. The complete amino acid sequence of the FR-patched RFB4 immunoglobulin. CDR's are boxed. Human framework amino acids that differ from that of the corresponding murine frameworks are in bold.

## (A) VH

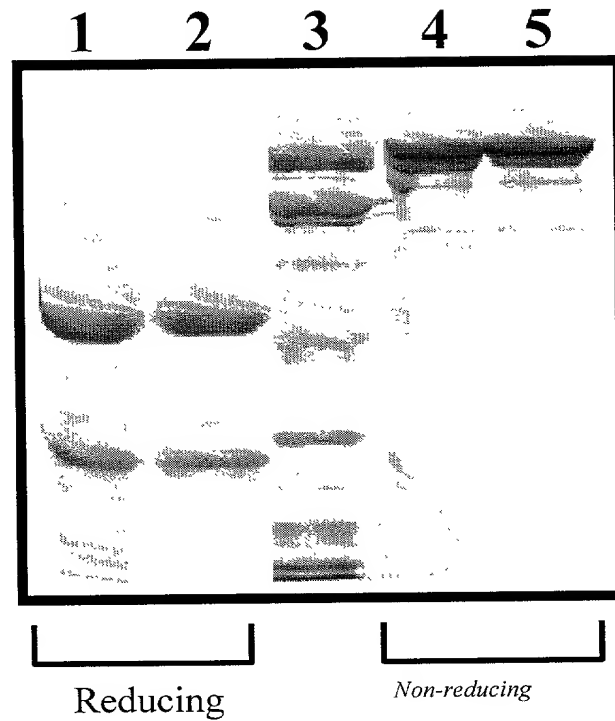
E V Q L V E S G G G L V **Q** P G G S L **R** L S C A A S G F S F S  
**I Y D M S** W V R Q A P **G K G** L E W V A **Y I S S G G G T**  
**T Y Y P D T V K G** R F T I S R D N A K N S L Y L Q M N S L  
**R V E D T A L Y Y C A R** **H S G Y G S S Y G V L F A Y** W G  
 Q G T L V T V S S

## (B) VL

D I Q M T Q **S P** S S L S A S V G D R V T I S C **R A S Q D I**  
**S N Y L N** W Y Q Q K P **G K A P** K L L I Y **Y T S I L H S**  
 G V P S R F S G S G S G T **E F T L T I S S L** **Q P E D F A T Y**  
 F C **Q Q G N T L P W T** F G G G T K V E I K

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**Figure 4.** SDS-PAGE analysis of purified cRFB4 and hpRFB4 under both reducing and non-reducing conditions.



1. cRFB4 (reducing)
2. hpRFB4 (reducing)
3. Size Marker
4. cRFB4 (non-reducing)
5. hpRFB4 (non-reducing)

**Figure 5. Flow Cytometry analysis on cRFB4 and phRFB4 specific binding to human Burkitt Lymphoma cell line, Raji cells.**

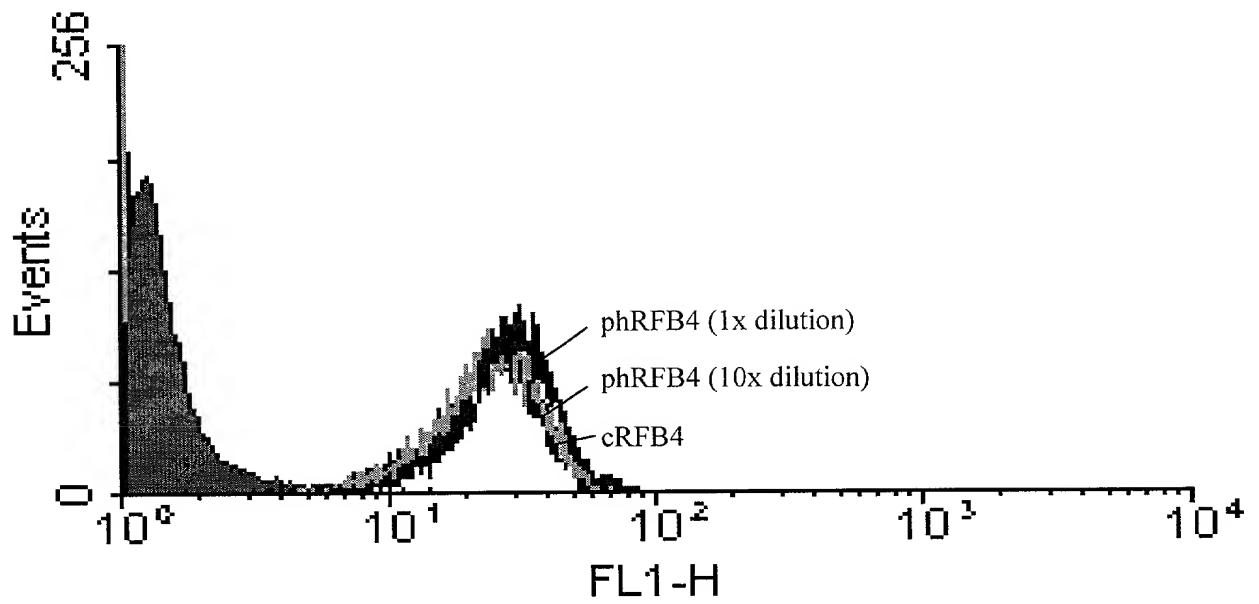
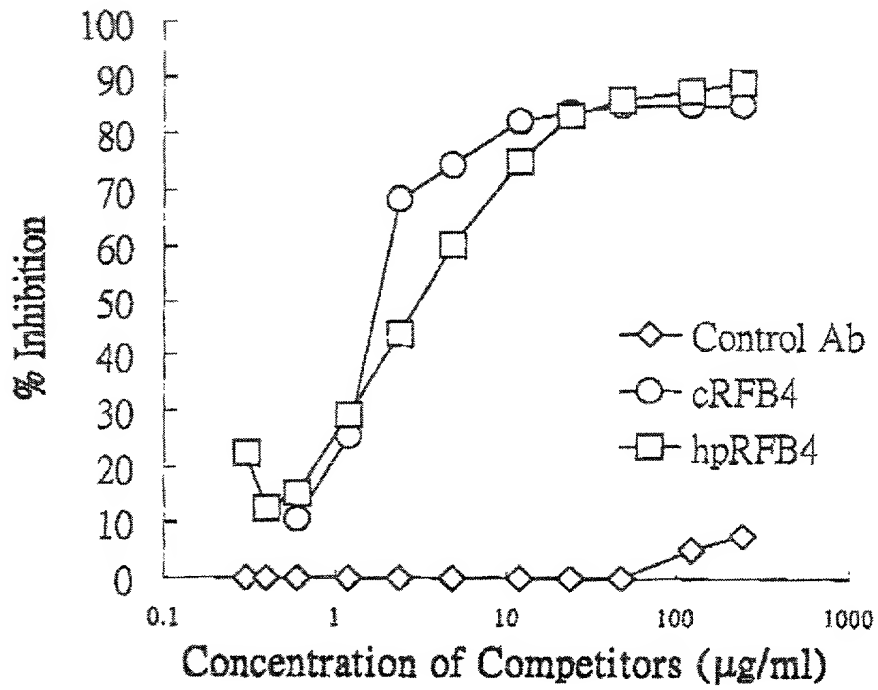


Figure 6. A competition binding assay comparing the specificity and affinities of cRFB4 and hpRFB4. An irrelevant antibody was used as a control.



**Figure 7.** The amino acid sequences for the variable region of the heavy chain (VH) (A), and the light chain (VL)(B) of the anti-CD20 antibody, 1F5. CDR's are boxed.

**(A) VH**

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Q V Q L R Q P G A E L V K P G A S V K M S C K A S G Y T F T
  S Y N M H   W V K Q T P G Q G L E W I G   A I Y P G N G D
T S Y N Q K F K G   K A T L T A D K S S S T A Y M Q L S S L
T S E D S A V Y Y C A R   S H Y G S N Y V D Y F D Y   W G Q
G T T L T V S S D

```

**(B) VL**

```

Q I V L S Q S P A I L S A S P G E K V T M T C   R A S S S L
S F M H   W Y Q Q K P G S S P K P W I Y   A T S N L A S   G
V P A R F S G S G S G T S Y S L T I S R V E A E D A A T Y F
C   H Q W S S N P L T   F G A G T K L E L K R

```



**Figure 8. A comparison of different human framework sequences to that of 1F5. Amino acid that differs from the parent framework is shown in bold. The source of the human framework is indicated in parenthesis on the left of each framework. CDR's are boxed**

## (A) VH

----- FR1 -----  
 Q V Q L R Q P G A E L V K P G A S V K M S C K A S G Y T F T  
 (LS2'CL) Q V Q L V A S G A E V N K P G A S V K V S C K A S G Y T F T

----- FR2 -----  
 S Y N M H W V K Q T P G Q G L E W I G A I Y P G N G D  
 (NEWM) W V R Q P P G R G L E W I G

----- FR3 -----  
 T S Y N Q K F K G K A T L T A D K S S S T A Y M Q L S S L  
 (783C'CL) R V T I T A D K S T S T A Y M E L S S L  
 (58'CL) R A T I S V D T S K N Q F S L N L S S V

----- FR3 -----  
 T S E D S A V Y Y C A R S H Y G S N Y V D Y F D Y W G Q  
 R S E D T A V Y Y C A R (4G12'CL) W G Q  
 T A A D T A V Y C C A R

----- FR4 -----  
 G T T L T V S S D  
 G T T V T V S S -

----- FR1 -----

R A S S L

----- FR2 -----

A T S N L A S

(WES)	G
(AND#)	G
(RZ)	G
(NOT)	G

----- FR3 -----

	V	P	A	R	F	S	G	S	G	S	G	T	S	Y	S	L	T	I	S	R	V	E	A	E	D	A	A	T	Y	F
(WES)	<i>V</i>	<i>P</i>	<b>S</b>	<i>R</i>	<i>F</i>	<i>S</i>	<i>G</i>	<i>S</i>	<i>G</i>	<i>S</i>	<i>G</i>	<i>T</i>	<b>E</b>	<b>F</b>	<b>T</b>	<i>L</i>	<i>T</i>	<i>I</i>	<i>S</i>	<b>S</b>	<b>L</b>	<b>Q</b>	<b>P</b>	<b>E</b>	<b>D</b>	<b>F</b>	<i>A</i>	<i>T</i>	<i>Y</i>	<i>F</i>
(AND#)	<i>V</i>	<i>P</i>	<b>S</b>	<i>R</i>	<i>F</i>	<i>S</i>	<i>G</i>	<i>S</i>	<i>G</i>	<i>S</i>	<i>G</i>	<i>T</i>	<b>D</b>	<b>F</b>	<b>T</b>	<i>L</i>	<i>T</i>	<i>I</i>	<b>T</b>	<b>S</b>	<b>L</b>	<b>Q</b>	<b>P</b>	<b>E</b>	<b>D</b>	<b>F</b>	<i>A</i>	<i>A</i>	<i>Y</i>	<i>F</i>
(RZ)	<i>V</i>	<i>P</i>	<b>S</b>	<i>R</i>	<i>F</i>	<b>T</b>	<i>G</i>	<i>S</i>	<i>G</i>	<i>S</i>	<i>G</i>	<i>T</i>	<b>D</b>	<b>F</b>	<b>F</b>	<i>L</i>	<i>T</i>	<i>I</i>	<i>S</i>	<b>S</b>	<b>L</b>	<b>R</b>	<b>P</b>	<b>E</b>	<b>D</b>	<i>V</i>	<i>A</i>	<i>T</i>	<i>Y</i>	<i>F</i>
(NOT)	<i>V</i>	<i>P</i>	<i>A</i>	<i>R</i>	<i>F</i>	<i>S</i>	<i>G</i>	<b>Y</b>	<b>N</b>	<i>S</i>	<i>G</i>	<b>N</b>	<b>S</b>	<b>A</b>	<b>F</b>	<i>L</i>	<i>T</i>	<i>I</i>	<b>N</b>	<i>R</i>	<i>V</i>	<i>E</i>	<i>A</i>	<b>G</b>	<b>D</b>	<b>E</b>	<b>A</b>	<b>D</b>	<i>Y</i>	<i>F</i>

---|-----FR4-----|

C	H Q W S S N P L T	F G A G T K L E L K R
C	(LSI'CL)	F G <b>G</b> G T K V E I K R
C	(NI)	F G V G <b>S</b> K V E <b>S</b> K R
C	(NIG-58)	F G A G T K L <b>T</b> V L R
C		

**Figure 9. The amino acid sequences for the FR-patched variable region of the heavy chain (VH) (A), and the light chain (VL)(B) of 1F5. CDR's are boxed. Human framework amino acids that differ from that of the corresponding murine frameworks are in bold. Murine frameworks that are retained in the FR-patched sequences are underlined.**

**(A) VH**

Q V Q L V A S G A E V N K P G A S V K V S C K A S G Y T F T  
S Y N M H W V **R Q P P G R** G L E W I G A I Y P G N G D  
T S Y N Q K F K G K A T L T A D K S S S T A Y M Q L S S L  
T S E D S A V Y Y C A R S H Y G S N Y V D Y F D Y W G Q  
 G T T V T V S S -

**(B) VL**

D I Q L T Q S P S S L S A S V G D R V T I T C R A S S S S L  
S F M H W Y Q Q K P G S S P K P W I Y A T S N L A S G  
 V P S R F S G S G S G T **E F T L T I S S L Q P E D F A T Y F**  
 C H Q W S S N P L T F G A G T K L T V L R

**Figure 10. Amino acid sequence of an alternative design of FR-patched variable regions for 1F5 (Alternative Design). CDR's are boxed. Human framework amino acids that differ from that of the corresponding murine frameworks are in bold.**

**(A) VH**

Q V Q L V A S G A E V N K P G A S V K V S C K A S G Y T F T  
 S Y N M H W V R Q P P G R G L E W I G A I Y P G N G D  
 T S Y N Q K F K G R V T I T A D K S T S T A Y M E L S S L  
 R S E D T A V Y Y C A R S H Y G S N Y V D Y F D Y W G Q  
 G T T V T V S S -

**(B) VL**

D I Q L T Q S P S S L S A S V G D R V T I T C R A S S S L  
 S F M H W Y Q Q K P G Q A P V P V I Y A T S N L A S G  
 V P S R F S G S G S G T E F T L T I S S L Q P E D F A T Y F  
 C H Q W S S N P L T F G A G T K L T V L R